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THE BACTERIOLOGY OF VAGINITIS *

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In the treatment and management of vaginitis in little girls there is a certain amount of confusion which arises from the uncertainty regarding etiology. Clinically, the affection resembles gonorrhea, and occasionally stained smears show typical gonococci. Certain cases are of known gonococcal origin, and many more are generally regarded as gonorrheal. The majority of cases, however, do not give the typical gonococcus picture with intracellular cocci. A positive gonococcal diagnosis from vaginitis smears is frequently based merely on the presence of forms which may actually be common pus cocci or even coccoid bacilli (colon bacilli). Such an error might be easily made from methylene blue stains and even from gram-stained smears if coccoid forms of the colon bacillus or if degenerated gram-negative staphylococci are present.

The question arises whether all these cases are primarily gonococcal. Even discharges of known gonococcal origin often continue, become less profuse, and of a serous character unlike ordinary gonorrhea. In such cases it is often assumed that the gonococcus has been replaced by other organisms, which have become parasitic in the less resistant vaginal mucosa following a primary gonorrhea.

The object of this work is to determine as far as possible whether vaginitis in children is gonococcal, and if so how long the gonococcus continues to be the specific exciting cause. My work consists in the bacterial examination of vaginitis by cultural and immunological methods, with special reference to the rôle played by the gonococcus. With this in view, the hypersensitiveness to and the immunity against known gonococcal products have been used in connection with the work.

The paper deals with a certain class of cases in the vaginitis wards of Chicago hospitals, with vaginal discharges diagnosed as gonococcal from the hospital smears. The patients varied in age from 15 months to 13 years and were largely from the poorer classes. Vaginitis was observed in four cases following rape and probably gonorrheal, in

* Received for publication May 25, 1914.

eighteen cases without known cause, and in five accompanying or following the exanthemata. The discharges varied in duration and amount, and for the most part had resisted treatment. Very few showed typical gonococcus forms by gram-stained and carefully examined smear. Vaginitis in such a class of cases is more suspicious of gonorrhea than in certain other classes, which are not to be considered here.

The complete aerobic bacteriology of fifteen of these cases was studied. The discharge was smeared over a hard human blood agar plate with one applicator, and then spread thinly with a second. The plates were examined in twenty-four and forty-eight hours, and colonies transferred to blood agar slants, and later identified culturally and microscopically. Aside from the gonococcus, the commonest were the staphylococcus, the colon bacillus, and the pseudodiphthericus bacillus. The latter, referred to as *B. pseudodiphthericus*, is a gram-positive bacillus, occasionally pleomorphic, which forms small transparent colonies on agar and blood agar, does not ferment dextrose, and some strains slightly alkalinize milk. It evidently corresponds to the organism designated by this name by other workers on vaginitis.

From one of the fifteen cases all three bacteria, the staphylococcus, the colon and pseudodiphthericus bacilli were isolated; from six, the pseudodiphthericus and the staphylococcus; from four, the pseudodiphthericus and the colon bacilli; from three, the staphylococcus; and from one, the colon bacillus without the others. Hemolytic strains of both the staphylococcus and the colon bacilli were the more common, but in a majority of cases both hemolytic and non-hemolytic strains of each were present. Other bacteria, chiefly from the streptococcus, proteus and acid-fast groups, were present in one, two or three cases. It is probable, as Curtis¹ points out, with reference to leukorrhea in women, that these aerobes are present only in the vulvar region unless frequent douching or other manipulation carries them deeper, where the vagina is otherwise infected only by anaerobes.

Five cases, with normal vaginas, were examined and the same three organisms, staphylococcus, colon bacillus and pseudodiphthericus bacillus were isolated; however, this number of cases is too small to give any idea of the frequency of their occurrence. Küster,² reviewing the work done on normal vaginal flora, shows the predominance

1. *Surg., Gynec. and Obst.*, 1914, 18, p. 299.

2. *Handb. d. path. Microorg.*, 1913, 6, p. 458.

of anaerobic organisms and points out that the pyogenic bacteria are present in but a small percentage of normal cases. Whether the larger proportion of our vaginitis cases, found to harbor these organisms, means that these bacteria are associated with the pathologic process, is a question. That they are not the sole exciting factor, and that they do not entirely replace the gonococcus, appears from the following.

Examinations for the presence of gonococci were also made by means of human blood agar plates. For this purpose the plates were incubated in a moist chamber at a temperature between 35 and 37 C. An ordinary desiccator jar, containing the plates over a little water and kept in the thermostat, was used successfully. After twenty-four hours, ten of the colonies resembling gonococcus were transferred to blood agar slants, and this was repeated after forty-eight, and sometimes seventy-two and ninety-six hours. The resulting growths were examined and identified. Gram stains were made of the same discharge, and records kept of the history and appearance of the same.

The isolation of the gonococcus was possible only by observing the precautions given above, and even then, in some cases, two, three, or even four repetitions of the work were necessary before the organism was isolated. From this, it seems probable that the gonococcus was present in even a larger number of cases than we found it. Similar examination of normal vaginal secretions revealed no organism resembling the gonococcus.

In the bacterial examinations of vaginitis discharges, twenty-seven were under cultural conditions favoring the growth of the gonococcus. After one or more attempts, gram-negative diplococci, which did not grow on plain agar, presumably the gonococcus, were isolated from twenty-two of the twenty-seven, though only fifteen of these strains could be kept in stock long enough for confirmation of their identity. These fifteen differed slightly from other stock gonococcal strains in that they seemed more susceptible to slight loss of moisture, though several were less susceptible to a rise of temperature to 42 C., for several hours. According to most authorities, reviewed by Koch,³ the gonococcus is killed in a few hours by a temperature of 40 to 41 C., though one, Wertheim, found that it could live at 42 C. Of all our gonococcal strains, none withstood this temperature save some of those isolated from vaginitis. They grew more scantily on media than most

3. Handb. d. path. Microorg., 1912, 4, p. 684.

strains from other lesions, but grew to some extent on plain agar after fewer transfers, some after only three. Koch finds that four or five generations on humanized media are usually necessary before growth on plain agar is possible.

These variations called for further assurance that they were strains of gonococcus, though some were from vaginitis cases almost surely of gonococcal origin. Thus, for example, Case 12, 13 years old, was admitted for vaginitis following alleged rape. There was a profuse discharge and microscopic examination showed it to be probably gonococcal. The organism was isolated on the first attempt. This strain, almost surely gonococcus, showed the same variations in susceptibility as those noted above. To confirm the identity of these several strains the complement-fixation test was used. Thick suspensions of the doubtful strains were made, heated to 60 C. for an hour, and used as antigen together with serum which had fixed complement with known gonococcus antigen. They all fixed complement, and were hence diagnosed certainly as gonococcus.

The number of gonococci which developed from the different discharges varied greatly. With approximately the same amount of material spread on each plate, some produced very many gonococcus-like colonies to the plate, others very few. There was no relation between the amount of discharge in a given case and the number of gonococci developing from the amount plated. From ten of the discharges there were a large number of gonococcus-like colonies to the plate, and of these ten, four were abundant, or moderate, and six scant. Of the other discharges, producing very few gonococcus colonies to the plate, ten were abundant and nine scant. From this it appears that the amount of discharge bears no relation to the number of gonococci present. A majority had but a slight discharge when examined, and in one the discharge had completely stopped. In the latter case the patient had been pronounced cured, and was about to be removed to a non-vaginitis ward when the gonococcus was first isolated.

The presence of gonococci after cessation of the discharge suggests that the original infection is responsible for the recurrences so frequently observed. Spalding⁴ reported twenty-six non-selected cases which she had been able to trace, and found that all but two had one or more recurrences of the discharge at intervals of three weeks to

4. *Am. Jour. Dis. Child.*, 1913, 5, p. 248.

six years. Others⁵ have thought it improbable that the gonococcal infection remains latent, and that a better explanation of these recurrences is reinfection with gonococcus, or infection with other organisms which find the mucosa a favorable ground for development due to the previous infection. From our results, latent infection would seem to be a probable factor.

Besides the cultural examinations, work was done to determine whether or not the gonococcus had excited the production of antibodies in these cases, and whether or not immunity reactions could be depended on as diagnostic of the presence of the gonococcus. The hypersensitiveness to known gonococcus toxins was tested by the skin reaction.⁶ Glycerin extracts of gonococcal protein introduced on a needle point at two places in the skin, caused zones of hyperemia which were not produced by two other control punctures. These were measured after twenty-four hours and their size noted and averaged. In gonococcal cases a large zone appears when the allergic curve is high, but a smaller zone or none at all when it is low. In non-gonococcal cases there may also be a small zone due to non-specific irritation of the toxin, especially to a very sensitive skin, or to anaphylactic bodies normally present. However, it seemed safe to consider cases positive (gonococcal) if the two zones averaged 5 mm. or together with a marked papule 4 mm., doubtful where they averaged 2 to 4 mm., and negative if less than 2 mm.

The power of the patient's serum to fix complement was also tested, together with antigen, consisting of known gonococcal suspension heated to 60 C. for an hour. If non-gonococcal, the serum is expected to give a negative test, though Smith⁵ finds that it may at times be weakly positive. The serum of a gonococcal case may be positive or negative depending on whether the immune curve is high or low when the blood is drawn. In our cases this curve apparently paralleled that of the cutaneous reactivity, for nine cases with positive skin tests also had sera which fixed complement, and two of the nine at another time did not give a positive skin reaction, nor a positive fixation test. A tenth case with a positive skin test was not tested for complement-fixation. The sera from cases giving a doubtful or negative skin test did not in any case fix complement.

5. Smith, *Am. Jour. Dis. Child.*, 1914, 7, p. 230.

6. Irons, *Jour. Infect. Dis.*, 1912, 11, p. 77.

The nature of this immune curve is not shown by our work, as this would involve frequent tests on an individual case. From the table following, however, it will be seen that most of the cases giving positive tests had discharges of less than three months' standing, while the negative results are largely from older cases. This would suggest that the curve is high in an early infection only, and falls after a few months. Naturally, the discharge also becomes less after a few months of treatment, but the immunity curve's decline did not depend on the decrease of the discharge apparently, for the latter was often quite active when the tests were negative.

The typical case seemed to be with a high immunity curve early. This soon decreases, followed later by a decrease in the discharge, and still later, if at all, by the elimination of the gonococcus. With Case 3, after seventeen days, both cutaneous and complement-fixation tests were positive and the discharge profuse. The discharge remained profuse for several weeks though the complement-fixation test became negative two weeks after the first test and remained negative, the cutaneous test being then doubtful. Case 22 was first examined more than four months after the beginning of the discharge. The cutaneous test was doubtful, each zone being red over 2 mm. diameter and red-dish over 4 mm. Several days later it was found to be fainter and after a month was quite negative, though there was still a scant discharge. Two months after this it was still negative, and the discharge had completely stopped, but at this time the gonococcus was isolated, and with known gonococcal serum it fixed complement. These seem to be typical cases. Only one case suggested that the curve may not follow this even decline. Case 24 still had a discharge of more than a month's duration when neither complement-fixation nor cutaneous tests were positive. Two weeks later, both were markedly positive, the discharge remaining about the same. From this case the gonococcus was not isolated. Here, the curve was late in reaching its height or was temporarily low when first tested.

Determinations made from stained smears seem to have no more constant value than those made from the immunity reactions. After Gram staining and careful examination of smears of forty-one discharge specimens, only seven were found to be clearly gonococcal, fourteen probably so, and twenty too doubtful for diagnosis. And yet almost all these cases were found to be gonococcal. Even a Gram stained smear can easily seem to be gonococcal, due to the failure of

the staphylococcus to hold the stain, as sometimes occurs, and to coccoid forms of gram-negative bacilli. Pus cells in these cases are usually few, and intracellular cocci much fewer, and so it is hard to be sure whether a slide is or is not gonococcal. The seven slides, called definitely positive, were those which contained intracellular gram-negative diplococci or extracellular gram-negative diplococci without the presence of rod forms. It is only such slides that we could depend on for proof of the presence of gonococci.

The definitely positive smears are chiefly of discharge from which large numbers of gonococcus colonies developed on the plates. On the ten examinations, in which very many gonococci were thus shown to be present in the discharge, only two of the stained smears were doubtful, four being definitely positive, and four probably positive. The large percentage of doubtful smears were principally from discharges containing relatively few gonococci. These points suggest that stained smears do not indicate the presence or absence of gonococci, but whether or not they are present in very large numbers. Perhaps if enough attempts were made, positive smears could be obtained from discharges having few gonococci; some portions of a discharge would have more organisms than others. Or some examinations might be made at a time of temporary increase in the number present, though we found no evidence of such temporary variations.

Hospital examinations of vaginitis smears usually yield a much larger proportion of gonococcal diagnoses. No criticism of such findings is intended, though many would be listed as doubtful in this report. In hospitals, our doubtful smears might be called positive, to avoid the danger of removing vaginitis cases to non-vaginitis wards. It is also to be borne in mind that these results are from a limited number of smears.

In vaginitis, diagnosis of the infective agent is important, and also the determination of the time of a cure. To determine the infective agent, all of the above mentioned laboratory tests are of value. Nearly half of our cases, on one or more tests, gave a definitely positive cutaneous reaction and nearly all of them a positive or doubtful one. Probably these were all positive at some time during the infection, but as such a time cannot be determined for ordinary diagnosis, the skin test's value is very limited. Neither can it be used to determine the time of clearing up of the infection, for the sensitiveness diminishes and frequently disappears before the infection does. The

same is true of the complement-fixation test, which does not even give a suggestive reaction unless the immunity curve is at its height. A negative complement-fixation test of the patient's serum has been regarded as evidence of cure of vaginitis.⁷ According to our cases it would have no such value, for the gonococcus was isolated from five cases with a negative fixation test at the time, and three of these still had quite an active discharge. The reactivity of the serum seems to decrease and usually to disappear within a few months, whether the gonococcus is still present or not. As slides also cannot be relied on, and as the discharge itself may cease before the infection does, we seem to be without means of diagnosing the cure of vaginitis.

A diagnosis of the infecting organism is made only with difficulty. Of this series of twenty-seven presumably gonorrheal cases, reliable diagnoses could have been made from the repeated cultural tests alone in twenty-two, from complement-fixation tests in nine (of those tested), from cutaneous tests in 10, and from repeated smear examinations in 5. The isolation of the organism would seem from the above to be the most reliable method, though if a case could be tested frequently from the time of infection the immunity tests would be a very great help. In most cases the value of slides was only confirmatory. Combinations of more than one of these methods would point out a large proportion of gonorrheal cases.

Following are lists of the cases on which these conclusions are based. The cutaneous tests are called positive where zones averaging 5 mm. or 4 mm. with marked papule appeared, doubtful where from 2 mm. to 4 mm. and negative where less than 2 mm. The entire series is thought to be gonococcal and from the first twenty-two the gonococcus was isolated. Where more than one examination are noted, the organism was isolated (except from Case 8) in the last.

From this analysis it may be concluded that vaginitis in this series of cases is largely and may be entirely gonococcal in origin, and that the gonococcus is present as long as the discharge continues. If there is a secondary invader it either cannot be easily cultivated aerobically, or it is a normal inhabitant of the vagina, and it does not altogether replace the gonococcus. The immunity to the gonococcus seemed to diminish after a few months, after which the infection frequently persisted. This immunity could be followed by complement-fixation or cutaneous tests. I found the latter more delicate as it gave a

7. Smith, *Am. Jour. Dis Child.*, 1913, 5, p. 313.

TABLE 1
LIST OF CASES FROM WHICH THE GONOCOCCUS WAS ISOLATED

Case	Previous Duration of Discharge	Amount of Discharge When Examined	Gon-Like Colonies to Plate	Careful Smear Diagnosis of Discharge	Cutaneous Test	Complement-Fixation Test
1.—	? over 3 days	Moderate discharge	Many	Probably positive	Positive	Positive
2.—	? several days	Scant discharge	Many	Probably positive	Not tested	Not tested
3.—1	17 days	Profuse discharge	Few	Doubtful	Positive	Positive
2	2 weeks later	Profuse discharge	Few	Probably positive	Doubtful	Negative
3	2 weeks later	Profuse discharge	Few	Doubtful	Not tested	Not tested
4	2 weeks later	Profuse discharge	Few	Doubtful	Doubtful	Negative
4.—1	18 days	Scant discharge	Few	Doubtful	Doubtful (4 mm.)	Negative
5.—1	19 days	Profuse discharge	Many	Positive	Doubtful (4 mm.)	Negative
2	12 days later	Moderate discharge	?	Positive	Doubtful	Negative
3	6 weeks later	Scant discharge	Many	Positive	Not tested	Not tested
6.—	2 months	Profuse discharge	Few	Probably positive	Doubtful	Not tested
7.—1	1 month	Scant discharge	Few	Probably positive	Positive	Positive
2	6 weeks later	Scant discharge	Few	Probably positive	Positive	Positive
8.—1	5 weeks	Moderate discharge	Many	Probably positive	Positive	Positive
2	3 weeks later	Moderate discharge	?	Positive	Positive	Positive
9.—	3 months	Scant discharge	Many	Positive	Positive	Positive
10.—	? over 3 months	Profuse discharge	Few	Probably positive	Positive	Positive
11.—	? over 3 months	Profuse discharge	Few	Probably positive	Doubtful	Negative
12.—	? few months	Profuse discharge	Many	Probably positive	Positive	Positive
13.—	? few months	Scant discharge	Many	Doubtful	Doubtful (4 mm.)	Positive
14.—	4½ months	Scant discharge	Few	Doubtful	Doubtful	Negative
15.—	? over 1 month	Scant discharge	Many	Positive	Doubtful	Not tested
16.—	? recent	Scant discharge	Few	Doubtful	Doubtful	Not tested
17.—	? recent	Scant discharge	Many	Doubtful	Positive	Not tested
18.—	? recent	Profuse discharge	Few	Doubtful	Doubtful	Not tested
19.—1	? unknown	Moderate discharge	Few	Doubtful	Doubtful (4 mm.)	Not tested
2	3 months later	Profuse discharge	Few	Doubtful	Negative	Not tested
20.—1	? unknown	Profuse discharge	Few	Doubtful	Negative	Not tested
2	3 months later	Scant discharge	Few	Doubtful	Negative	Not tested
21.—1	? few months	Scant discharge	Few	Doubtful	Negative	Not tested
2	2 months later	Scant discharge	Few	Doubtful	Doubtful	Not tested
22.—1	Over 4 months	Scant discharge	Few	Probably positive	Doubtful	Not tested
2	1 week later	Scant discharge	?	Doubtful	Doubtful	Not tested
3	1 month later	Scant discharge	?	Not examined	Doubtful	Not tested
4	3 months later	No discharge	Few	Doubtful	Negative	Not tested

TABLE 2
LIST OF CASES FROM WHICH THE GONOCOCCUS WAS NOT ISOLATED

Case	Previous Duration of Discharge	Amount of Discharge When Examined	Careful Smear Diagnosis of Discharge	Cutaneous Test	Complement-Fixation Test
23.—	? few weeks	Scant discharge	Probably positive	Positive	Positive
24.—1	? few weeks	Profuse discharge	Positive	Doubtful	Negative
2	2 weeks later	Profuse discharge	Doubtful	Positive	Positive
25.—1	3 months	Profuse discharge	Probably positive	Doubtful	Negative
2	6 weeks later	Moderate discharge	Doubtful	Doubtful	Negative
26.—	? recent	Moderate discharge	Doubtful	Doubtful	Not tested
27.—1	? unknown	Scant discharge	Doubtful	Doubtful	Not tested
2	3 months later	Moderate discharge	Doubtful	Negative	Not tested

suggestive reaction when the immunity was too weak for complement-fixation. The cases giving a definitely positive test with one did with the other also. Definite diagnoses from smears seemed of value only in the few cases when carefully examined Gram preparations showed the typical gonococcal picture, while a majority of slides could not be called positive unless confirmed by other signs. While laboratory methods are of assistance in determining the nature of the infecting organism, none of them seems to be of value in defining the time of its elimination.

I wish to thank Prof. E. O. Jordan for suggesting this work in partial fulfillment of the requirements for the degree of M.A. at the University of Chicago; Dr. Ernest E. Irons for help throughout the work, and Dr. Homer K. Nicoll, who made the complement-fixation tests.